World Agroforestry Centre / Soil-Plant Spectral Diagnostics Lab	**Code: METH07V01**	**Date: July 31, 2014** **Author: Dickens Ateku**

STANDARD OPERATING PROCEDURES

MID-INFRARED DIFFUSE REFLECTANCE SPECTROSCOPY METHOD FOR ANALYSING SOILS USING BRUKER ALPHA SPECTROMETER

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SCOPE AND APPLICATION

This is a World Agroforestry Centre's Soil-Plant Spectral Diagnostic Laboratory SOP for analysis of soils using DRIFT mid IR spectra recorded with Alpha R module. The technique involves shinning of mid infrared light on the samples and capturing of the diffused and reflected light in to raw file known as the interferogram. The recorded spectrum bears the qualitative and quantitative chemical aspect of the sample for further use with various data processing systems. This SOP is applicable to those doing analysis on infrared laboratory using ALPHA spectrometer Module using either the Potassium Bromide (KBr) or Zinc Selenide (ZnSe) beam splitter. The spectrometer is operated using the OPUS software.

PRINCIPLE

The ALPHA R Module has the DRIFT accessory designed for measurements in diffuse reflection. The optics of a diffuse reflection accessory is designed in such a way that detection of diffuse reflected light is optimized and the detection of specular reflected light is minimized. A DRIFT is an analyzing technique in FT-IR spectroscopy that makes use of the phenomenon of diffuse reflection. The key advantage of these techniques is that it enables analysis of strongly scattering and absorbing samples unlike transmission measurements and equally has high signal intensity.

ABBREVIATIONS AND DEFINITIONS

DRIFT –Diffuse Reflectance Fourier Transform spectroscopy.

OPUS –Bruker's Optical User Software

SOP –Standard Operating Procedures

MIR – Mid Infra-Red

STD – standard soil sample

RELATED DOCUMENT

- ALPHA spectrometer Technical manual
- Sample reception and preparation SOP
- MIR workflow
- Infrared health and safety

RELATED FORMS

- Login form
- Recording sheet

SAFETY AND ENVIRONMENT

- Electrical hazards: Electrical systems must conform to the ICRAF standards. No shock hazards exist inside the instruments. Do not try to repair the faulty electrical system but call for assistance from an authorized service representative or an individual with training in electronic repair. The instruments require a third-wire protective grounding conductor. Three-to-two wire adapters are unsafe for these instruments.
- Refer to the safety instructions in the equipment manual available in the lab.

REQUIREMENTS

- **Supplies**
  a. 70% Ethanol (for cleaning the Aluminium sample holding cups)
  b. Ear buds (for cleaning the Aluminium sample holding cups)
  c. Tissues

- **Equipment**
  a. Retsch motor grinder RM 200
  b. Aluminium sample holding cups
  c. Micro spatula

PROCEDURE

- **Soil Sample loading**

The loaded samples should be air-dried sieved through 2mm sieve, subsampled using the coning and quartering method and grinded to less than 0.5mm (fine powder particle size between 20- 53 µm) using RM 200 Restch motor grinder (Refer to the Alpha Spectrometer Technical Manual pages 10 and 11).

a. Load the sample into aluminium sample cup by filling it with the fine ground soil. It takes about 1-2 g depending on the type of soil. (Refer to the Alpha Spectrometer Technical Manual page 12).
b. Remove the excess soil and level surface with a straight-edged tool such as a straight spatula, glass slide or small knife.
c. Place the loaded samples in the sample holder tray which accommodates 20 samples.
d. Prepare a data sheet indicating sample identifications (IDs) and sample position in the loading tray. Ensure that you enter the sample ID immediately after filling the cup

- **Recording spectra measurement in OPUS**

a. Power up the instrument and log into the OPUS software (Technician must get himself/herself familiarized with operating instructions of the instrument by going through the ICRAF soil and plant spectral diagnostic laboratory adopted version of user manual for Alpha R Module).Give the instrument about 15 minutes to stabilize.
b. Select the advance data collection option in OPUS and load the ICRAF soil and plant diagnostics laboratory adopted method (ICRAF *Drift.xpm*.) refer to Alpha spectrometer technical manual.
c. Create a folder that identifies the batch of soils being analyzed on the computer connected to the instrument. All spectra recorded on test samples should be saved in a separate folder. For example C:\ICRAF\RAW\SOTUBA SITE SOILS…….for all Sotuba soils. Refer to SOP.

d. Ensure that the instrument is moisture free by adequately generating the Desiccants at 105 degrees centigrade for 1 hour (Refer to the Alpha Technical manual).Give the instrument 15 minutes to stabilize after changing the desiccants.
e. Always monitor the instrument moisture, record the moisture level at the start and end of a run on the recording sheet.
f. Optimize the Alpha ADC count (signal position and amplitude count) with the gold reference cup and save the value (record the result value on the recording sheet).
g. For the standard used, the optimized Alpha signal should not be saved, just make sure you have recorded the result value on the recording sheet. (Always confirm that the correct soil standard is the one loaded on the sample cup before taking any measurements).
h. Take **five** replicate scans of the soil standards used in the morning before scanning the samples and in the evening after scanning the samples. (Create a new folder for these daily standards).
i. Use Alpha sample trays consistently when loading making sure the correct sample loading protocols are followed.
j. Before scanning the sample cup, check to make sure that it was loaded well.
k. If there are rejected scans when scanning the sample, repeat the whole scanning procedure a fresh after checking if the sample was loaded well.(if this persists make a note on the “comments” section of the recording sheet concerning the problem).
l. Enter the sample serial number (SSN) on sample description menu and click on the “sample single channel” tab to scan the sample
m. Open the sample compartment and replace the scanned sample with the next sample on the sample holder and scan.

QUALITY CONTROL

- **Reference Material**

  a. Reference standards: Infrared ICRAF laboratory uses Mua soil and White sand as reference standards. Everyday a new spectrum is recorded on these samples before analyzing test samples and checked against the previous records. Appropriate correction actions where necessary are implemented. Normally no adjustments are required.

  b. Blanks: A standard gold cap is sued to correct the background

  c. Calibration: Polystyrene internal calibration

- **Data Validation**

  a. Check the sample data and QC data to verify that the data to be reported is based on acceptable analyses and meet acceptable limits.

  b. Verify that the soil standard spectra have been entered into the appropriate logs.

  c. Verify that the results are saved in appropriate folder and format.

  d. Place data in the laboratory database.

- **Supervisor Review**

  a. Check the sample data and QC data to verify that the data to be reported is based on acceptable analyses and meet acceptable limits

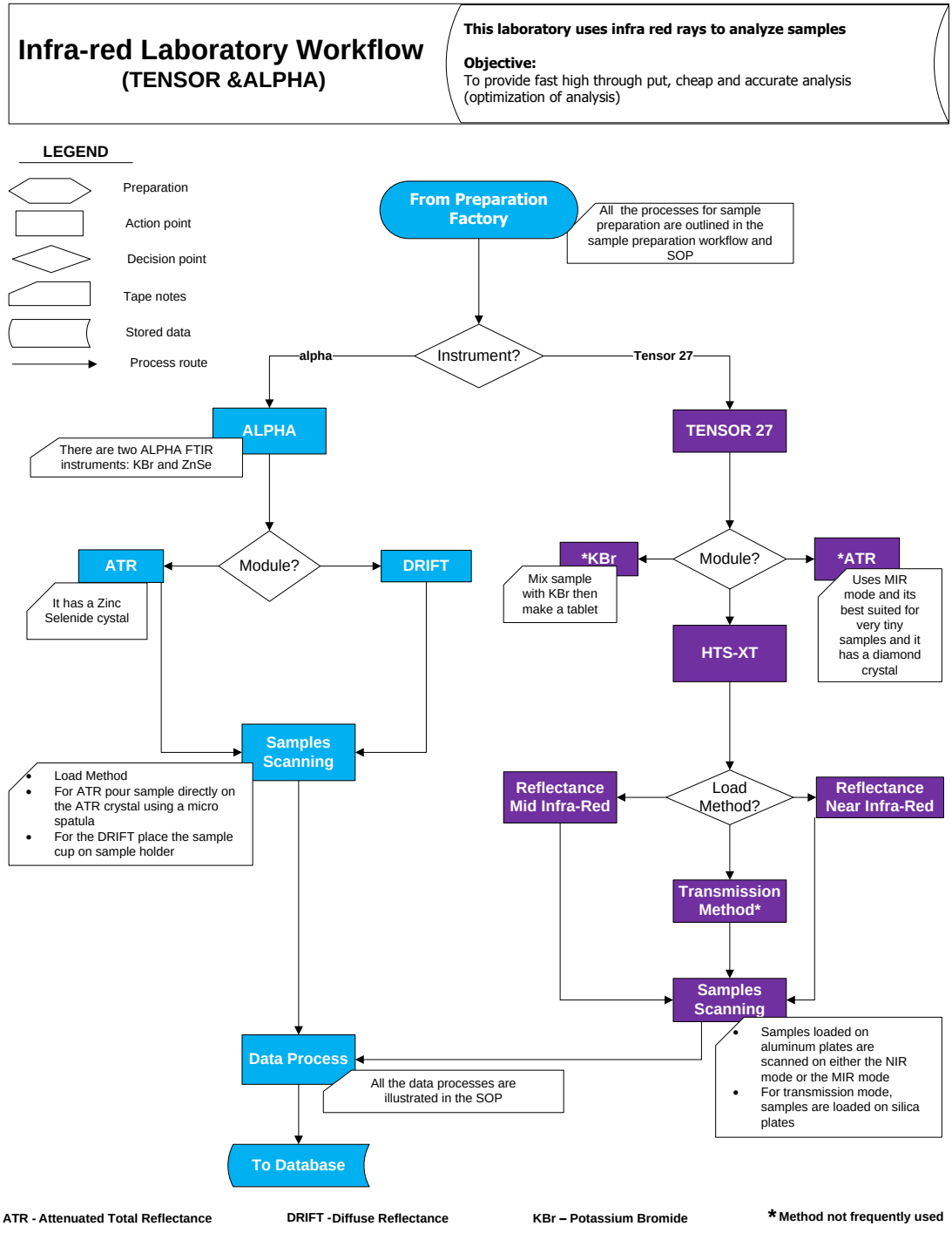
  b. If any errors are found, return data for repeat analysis.

REFERENCES

- ALPHA – R Module for diffuse Reflection Measurement, 1st Edition 1009974, BRUKER OPTICS, May 2008, page 1–page 9
- ALPHA Installation Instructions, 1st Edition 1005543, BRUKER OPTICS, April 2007, page 1–page 35

ANNEX

1. Work Flow


Infra-red Laboratory Workflow
(TENSOR &ALPHA)
This laboratory uses infra red rays to analyze samples
Objective:
To provide fast high through put, cheap and accurate analysis (optimization of analysis)
LEGEND
Preparation
Action point
Decision point
Tape notes
Stored data
Process route
From Preparation Factory
All the processes for sample preparation are outlined in the sample preparation workflow and SOP
Instrument?
alpha
Tensor 27
ALPHA
There are two ALPHA FTIR instruments: KBr and ZnSe
TENSOR 27
Module?
ATR
It has a Zinc Selenide cystal
DRIFT
*KBr
Mix sample with KBr then make a tablet
Module?
*ATR
Uses MIR mode and its best suited for very tiny samples and it has a diamond crystal
HTS-XT
Samples Scanning
Load Method
For ATR pour sample directly on the ATR crystal using a micro spatula
For the DRIFT place the sample cup on sample holder
Reflectance Mid Infra-Red
Load Method?
Reflectance Near Infra-Red
Transmission Method*
Samples Scanning
Samples loaded on aluminum plates are scanned on either the NIR mode or the MIR mode
For transmission mode, samples are loaded on silica plates
Data Process
All the data processes are illustrated in the SOP
To Database
ATR - Attenuated Total Reflectance
DRIFT -Diffuse Reflectance
KBr – Potassium Bromide
* Method not frequently used

2. Infrared ALHPA spectrometer standards fact sheets

ALPHA Instrument calibration standards

These are standards used for all analysis in the ICRAF Soil-Plant diagnostic laboratory.

- Heavy black cracking clay soils (HBCCS), Katumani, and MUA standards are run every day in the morning before you start any analysis and every time you start on a new site.
- White sand a commercial Standard which is run every day in the morning before you start any analysis and every time you start on a new site.

3. Quality Control fact sheet

PROCESS	DESCRIPTION
Background Measurement	The instrument is set in a way that it forces the user to perform Background measurement after every 5 minutes. The measurement normally increases the signal to noise ratio of the spectrum produced (improves the quality of the spectra)
Measurement of the selected soil standards.	The standards used in ICRAF infrared laboratory are HBCCS, Katumani, MUA and White Sand. The measured spectra are used to monitor the performance of the instrument.
Check on the quality of the spectrum measured	Always monitor the instrument moisture, record the moisture level at the start and end of a run on the recording sheet. Ensure that the spectrum measured does not have any noise due to moisture and carbon dioxide. The moisture noise peaks are expected at around 3600cm and 1600cm wave numbers while the

	carbon dioxide peak is expected at around 2400cm wave numbers.